Released by/Date Effective:

Author Name	Title	Signature/Date

Approver Name	Title	Signature/Date

1. PURPOSE

1.1. The purpose of this procedure is to use the GeneQuant Calculator to measure bacterial cell culture solutions in order to optimize induction and harvest times for bacterial plasmid production.

2. SCOPE

2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility, Room C2007.

3. REFERENCES

3.1. HSL_LAB_008.01: GeneQuant Use and Maintenance Form

3.2. HSL_LAB_008.02: Bacterial Cell Culture Measurement Form

3.3. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

3.4. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory

3.5. HSL_GL_005: Signature and Initial Identification System

3.6. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory

3.7. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory

3.8. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory

3.9. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures

3.10. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory

3.11. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance

3.12. HSL_EQ_005: Use and Maintenance of a Molecular Devices M5 Plate Reader in the HPV Serology Laboratory

3.13. HSL_EQ_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory

3.14. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory

3.15. HSL_EQ_009: Use and Maintenance of the Liquid Nitrogen Freezer

3.16. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory

3.17. HSL_EQ_019: Use and Maintenance of the Milli-Q Integral 3 Water System

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.

4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. REAGENTS, CHEMICALS AND EQUIPMENT

5.1. Certified class II biological safety cabinet

5.2. -80˚C freezer (Temperature range: -65°C to -85°C)

5.3. Pipettes (Ranging from 2 µL to 1000 µL)

5.4. 2-8°C Refrigerator

5.5. Growth media

5.6. Orbital Shaker

5.7. GeneQuant Calculator

5.8. M5 Microplate Reader

5.9. Bleach (Warehouse, Cat # 68100251)

5.10. Ster-ahol (VWR, Cat # 14003-354)

5.11. Cavicide (Warehouse, Cat # 79300360)

5.12. Brand Tech Cuvettes (Thomas Scientific, Cat # 1213M46) or equivalent

5.13. Calibration check filter set (part number 80-2109-88)

6. HEALTH AND SAFETY CONSIDERATIONS

6.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

6.2. Refer to the respective SDS when working with any chemicals.

6.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

7. DEFINITIONS

Term	Definition
ATRF	Advanced Technology Research Facillity
FME	Facilities, Maintenance and Engineering
HPV	Human Papillomavirus
HSL	HPV Serology Laboratory
SDS	Safety Data Sheets
SOP	Standard Operating Procedure

8. INSTRUMENT OPERATION

8.1. Turn on instrument and wait for instrument to display "Instrument Ready".

8.2. There are 28 keys on the keypad, organized into 3 areas; Numeric Keypad, Utility Keys and Reading keys.

8.3. The following Utility Keys are available for use on the instrument.

8.3.1. **Set up:** Enables configuration for the various instrument functions Pressing set up after the appropriate reading key will activate the set-up process for that mode, with the first option being highlighted.

8.3.2. **Cal Check**: Enables instrument to be tested for absorbance accuracy, wavelength accuracy and stray light with special filters.

8.3.3. **Select**: Enables review of options in the set-up parameters and viewing results for base sequence and nucleic acid modes.

8.3.4. **Print**: Outputs data to printer or PC via multipurpose output port.

8.3.5. **base seq**.: Enables entry of a primer or oligonucleotide base sequence for calculation of Theoretical Absorbance, Molecular Weight and conversion factor.

8.3.6. **Tm**: Enables determination of Tm (from Abs260) and display of calculated Tm for an entered base sequence.

8.3.7. **Stop**: Behaves as an "escape" key, moving the user back to "Instrument Ready".

8.3.8. **Set Ref**: Reads reference values at appropriate wavelengths.

8.3.9. **Enter**: Enter / accept option in set up, and acts as reading key.

8.4. There are 7 Reading Keys; however, only the following will be used for this procedure.

8.4.1. **Abs**: Measures absorbances at 230, 260, 280, 320, 595, 600 nm.

8.4.2. **Cell culture**: Measures optical density at 600 nm, calculates corrected OD on individual samples.

8.4.3. **Protein 280 meas**: Measures absorbances at 595, 546 or 562 nm. Calculates protein concentrations on individual samples.

8.5. The instrument should be calibrated annually or if concerns are noted by the analyst.

8.5.1. Using a calibration check filter set, the instrument can be checked for absorbance accuracy at 230, 260, 280, 320, 595 and 600nm, wavelength accuracy at 260 and 280nm and stray light at 260nm.

8.5.2. An accredited service engineer with your supplier should have a set of filters (secondary standards traceable to NIST), and be able to verify the instrument performance; a certificate for record keeping will be provided. A calibration check filter set is available for periodic testing of the instrument to ensure that it is working to specification if you prefer to do it in house; instructions for carrying this out are provided with the filters.

8.5.3. Observe all necessary precautions if dealing with hazardous samples or solvents.

Note: If the calibration check filters are not available, a parallel check using a reagent such as BSA can be performed. To do this, the analyst must compare like with like, using the same sample with the same cell, and obtain absorbance measurements on both at the same wavelengths. GeneQuant has a bandwidth of 5 nm, and this should be compared with a spectrophotometer having the same bandwidth, since this parameter affects absorbance values.

8.6. Clean up any spills immediately, with the appropriate cleaner, such as cavicide or 10% bleach solution. Ster-ahol may only be used as a secondary cleaner.

9. BACTERIAL CELL CULTURE MEASUREMENT

Note: GeneQuant has smaller optics than most conventional spectrophotometers, and more scattered light is transmitted through to the detector resulting in lower than expected OD 600 values. Results obtained by comparing measured OD 600 with expected OD 600 indicate that a correction factor of 2.0 is required to make the data comparable to larger instruments; this factor is included as a default value in set up.

9.1. Insert blank into instrument and press “set ref” button. Remove reference from the instrument. (Blank will be the media used for culture)

9.2. Add 100 µL of well mixed bacterial sample into the cuvette.

9.3. Insert cuvette into the instrument and press enter or “cell culture” button.

9.4. The OD at 600 nm is measured and displayed together with the corrected result.

9.5. Record the OD 600 (not the corrected result) on Form HSL_LAB_004.02.

9.6. In the event that “HSL_LAB_004: Plasmid Purification Using a QIAGEN HiSpeed Plasmid Maxi Kit” is not being used in conjunction with this procedure, record the assay information on HSL_LAB_008.02.

10. SYSTEM SUITABILITY CRITERIA

10.1. The reference should read at “0” to indicate there is no bacterial cell growth.

11. DATA ACCEPTANCE CRITERIA

11.1. The OD of the bacterial cell is expected to be above the value of the blank.

12. ATTACHMENTS

12.1. Not applicable.

13. REVISION HISTORY

Revision Start Date	Version #	Changes	Reasons
16Oct17	New	Create new SOP describing the use of the GeneQuant Calculator to track bacterial cell culture growth.	New SOP.

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure
GeneQuant Use and Maintenance Form		
Form ID: HSL_LAB_008.01 Document ID: HSL_LAB_008	Version 1.0	Page 1 of 1

Equipment ID:

Date	Initials	Disinfectant(s) Used/ Lot Number	Activity Performed
		☐ N/A ☐ Cavicide, Lot #: ☐ N/A ☐ Ster-ahol, Lot #:	☐ Clean Instrument ☐ Other:
		☐ N/A ☐ Cavicide, Lot #: ☐ N/A ☐ Ster-ahol, Lot #:	☐ Clean Instrument ☐ Other:
		☐ N/A ☐ Cavicide, Lot #: ☐ N/A ☐ Ster-ahol, Lot #:	☐ Clean Instrument ☐ Other:
		☐ N/A ☐ Cavicide, Lot #: ☐ N/A ☐ Ster-ahol, Lot #:	☐ Clean Instrument ☐ Other:
		☐ N/A ☐ Cavicide, Lot #: ☐ N/A ☐ Ster-ahol, Lot #:	☐ Clean Instrument ☐ Other:

Comments:

☐ N/A

Review By/Date:	

Reagents

Reagent	Lot Number	Expiration Date
Growth Media:	☐N/A	☐N/A
☐N/A	☐N/A	☐N/A

Equipment

Equipment Description	Equipment ID	Calibration Due Date
☐N/A BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐Other:	
☐N/A -80°C Freezer	☐ HSL_022 ☐Other:	
☐N/A Orbital Shaker	☐ HSL_011 ☐Other:	
☐N/A 2-8°C Refrigerator	☐HSL_029 ☐Other:	
☐N/A -20°C Freezer	☐HSL_034 ☐Other:	
☐N/A M5 Microplate Reader	☐HSL_018 ☐Other:	
☐N/A Pipette: µL		
☐N/A Pipette: µL		
☐N/A Pipette: µL		

Sample Identification

Sample 1 ☐N/A	
Sample 2 ☐N/A	
Sample 3 ☐N/A	
Sample 4 ☐N/A	

Comments:

☐N/A

Performed By/ Date:	
Reviewed By/ Date:	

Attachments/Notes

Comments:

☐N/A

Performed By/ Date:	
Reviewed By/ Date:	